Effect of Metabolizable Undegradable Protein and Supplemental Methionine and Lysine on Production Parameters and Nitrogen Efficiency of Holstein Cows in Early Lactation

Undergraduate Honors Research Project

2001
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Abstract

Milk production can have a negative effect on the environment from excessive excretion of nitrogen (N) by dairy cows. We hypothesized that targeted dietary changes to the N-intake pools, or the N available in the feed, would improve N efficiency by dairy cows, thus reducing negative environmental impact from milk production. Forty multiparous and 22 primiparous Holstein cows were used in a 2x2 factorial arrangements of dietary treatments to determine the effects of (1) metabolizable rumen undegradable protein (**M-RUP**): 100% (**LoM-RUP**) or 110% (**HiM-RUP**) of the requirements stated by the National Research Council (NRC) (2001), and (2) methionine (**Met**) and lysine (**Lys**) supplementation: control levels of 6.15 and 1.80% Lys and Met, respectively (**LoAA**), or supplementation at 108% and 120% (**HiAA**) of control levels for Lys and Met respectively. The Lys to Met ratio in the HiAA diets was set at 3.0, as recommended by the NRC (2001), whereas the LoAA diets contained Lys to Met ratios of 3.3 to 3.4. Cows were randomly assigned to one of four dietary treatments 14 to 21 days postpartum and continued on their assigned diet for 12 weeks. There was no effect of treatment on dry matter intake or milk yield. Treatment had significant effects on milk true protein (2.96, 3.08, 2.97, 3.02; SE = 0.041 %), milk fat (3.21, 3.07, 3.34, 3.39; SE = 0.11 %), and lactose (4.81, 4.73, 4.90, 4.78; SE = 0.024 %) for the HiM-RUP-LoAA, HiM-RUP-HiAA, LoM-RUP-LoAA, and LoM-RUP-HiAA treatments respectively. Lower levels of M-RUP significantly decreased urinary N excretion. Cows were more efficient at converting intake N to milk N at higher amino acid levels and with a Lys to Met ratio at 3.0.

Dietary manipulation of N fractions can reduce the impact of intensive dairy production on the environment.

Acknowledgements

I would like to thank Susan Noftsger for her endless advice and help with this project. This trial would not have gone as smoothly without her. I would also like to thank Dr. Normand St-Pierre for giving me the chance to do this project and for the countless things I have learned from him over the past year. Thank you to Dr. Jeffrey Firkins for encouraging me to pursue an honors project. Finally, I would especially like to thank my parents, Robert and Karen, my brother, Jake, and my sister-in-law, Jennifer for all their support and optimism that helped me get through this project.

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Abbreviation Key

ADF- Acid detergent fiber

CP- Crude protein

DM- Dry matter

DMI- Dry matter intake

M-RUP- Metabolizable-rumen undegradable protein

MUN- Milk urea nitrogen

NDF- Neutral detergent fiber

NRC- National research council

NEL- Net energy of lactation

RDP- Rumen degradable protein

RUP- Rumen undegradable protein

Introduction

Ammonia losses from manure are thought to account for over 90% of the ammonia produced from agricultural operations and 45% of that is contributed by cattle enterprises (Meisinger, 2000). Excess N entering the environment in the form of ammonia and nitrates leads to pollution of air, nearby lakes, streams, and coastal waters. Ammonia is converted from urea, which is excreted in the urine and feces of livestock animals. Over-feeding of crude protein (**CP**) in the dairy industry in an effort to increase milk yield has lead to an increase in N excretion in the form of urea (St-Pierre and Thraen, 1999).

Feeding strategies can lower urinary urea N. Increasing the growth of rumen microbes by synchronizing carbohydrate and protein degradation can increase nitrogen efficiency because the amino acid profile of microbial protein is similar to the relative requirements in essential amino acid (**EAA**) needed by high producing cows (NRC, 2001). However, the EAA profile of microbial protein may be deficient in methionine (**Met**) and lysine (**Lys**) (Richardson and Hatfield, 1978). Therefore, ruminally protected forms of Met and Lys need to be included in dairy rations to create an optimal EAA profile.

Another way to decrease urinary urea N is to feed rumen undegradable protein (**RUP**) that can bypass the rumen relatively unchanged and provide specific amino acids directly to the intestine of the animal. In this way, cattle can be fed the specific EAA profile to meet their needs, which means that this protein will be converted to milk protein more efficiently than other forms of CP. At equal N intake, the higher the conversion of absorbed N to milk N and less of absorbed N is converted to urinary urea N. Increasing the efficiency of N utilization for milk production would not only decrease urea production and degradation but could also increase the nutritional and economic value of the milk by increasing milk protein and fat yields.

The purpose of this study was to determine whether lowering metabolizable-rumen undegradable protein (**M-RUP**) would lead to increased N efficiency, and to determine if supplementation of metabolizable Lys and Met in the proper ratio would lead to increased milk yield or component production.

Literature Review

Lysine and Met are generally considered to be the two most limiting amino acids for dairy cattle. Schwab et al., (1992), infused Holstein cows with Met, Lys, and a combination of Met and Lys to determine which was the first limiting amino acid during early and mid-lactation. In early lactation, Lys was the most limiting amino acid and Met was the second-limiting amino acid for milk protein synthesis. However, during mid-lactation it was not clear whether Lys was first limiting or whether Lys and Met were co-limiting.

Rumen undegradable protein has the ability to supply specific amino acids directly to the intestine of the animal. Feeding properly balanced M-RUP sources has the potential to increase the efficiency with which amino acids are utilized. The following studies have looked more closely at the effects of different levels of RUP on N efficiencies and milk and component production. Wright et al. (1998) fed RUP supplements from animal products at 4.5, 14.9, and 29.1% of dry matter intake and found that urinary N and fecal N outputs increased at higher levels of RUP and found that overall N efficiency (N utilization for milk) decreased. The higher RUP levels did increase milk yield, milk true protein production, and lactose production but decreased milk fat production. Cunningham et al. (1996) looked at different levels of dietary RUP from soybean products. Their results agreed with the findings of Wright et al. (1998) that increased levels of RUP increased milk yield and milk true protein production. However, contrary to the observation of Wright et al. (1998), Cunningham et al. (1996) saw an increase in milk fat production at higher RUP levels. These conflicting results could be due to different amino acid profiles found in the RUP sources. Research has shown that the relative concentration of dietary amino acids may have a greater impact on milk components and N efficiency than dietary CP levels. Bach et al. (2000) fed diets containing 18 and 15% CP in combination with high and low essential amino acid profiles. The high amino acid profile was comparable to that found in casein. They observed that the amino acid profile had a greater effect on milk, protein, and fat production than did concentration of CP. They also found that an improvement in N efficiency was associated with a higher amino acid profile.

Many studies have looked at the effects of feeding supplemental rumen protected Lys and Met on milk and component yields. However, the results from these studies have been variable, possibly due to ration ingredients rather than protein or amino acid level. Several studies (Donkin et al., 1989; Colin-Schoellen et al., 1995; Piepenbrink et al., 1996; Nichols et al., 1998) have shown no affect of supplemental rumen protected Lys and Met on milk yield with corn-based diets. Bateman et al. (1999) also reported no affect of supplemental rumen protected Lys and Met on milk yield. In this study, the basal diets were balanced for 15.5% CP. Supplemental CP was then added in the form of soybean meal, urea, or a mixture of fish and blood meal to bring the CP level up to 18% of diet DM. Results indicate that, at high CP levels, Lys and Met requirements were being met and additional Lys or Met has no beneficial impact on production.

Other studies have shown an increase in milk yield due to Lys and Met supplementation. Robinson et al. (1995) found that supplementation increased milk yield. The basal diet used in the study was balanced to meet CP requirements.

Thus, either the Lys and Met were still limiting even though the CP requirement was met or else Lys and Met can enhance milk yield when fed above requirement levels. Xu et al. (1998) showed that very high levels of Lys and Met can have a beneficial effect on milk yield. They added 40 g/d of Lys and 13 g/d of Met to a ration already balanced for 112 and 103% of required metabolizable Lys and Met, respectively, found a significant increase in milk yield from the Met and Lys supplementation. This supports that theory that Lys and Met may have an effect on milk yield when fed above their calculated requirements.

Results on the effect of RUP Lys and Met supplementation on milk fat percentage and yield are variable. Several studies (Colin-Schoellen et al., 1995; Robinson et al., 1995; Xu et al., 1998) showed an increase in milk fat percentage when additional RUP Lys and Met were fed. However, these studies did not look at which amino acid, Lys or Met, was responsible for the increase in fat percentage. When RUP Met was the only supplemental amino acid fed, Overton et al. (1996) showed a significant increase in milk fat yield and percent. When Met was calculated as being the sixth limiting amino acid, Robinson et al. (1998) reported a numerical increase in milk fat production from supplemental RUP Met. This indicates the Met can increase milk fat even when it is not calculated as being limiting. It is unclear why Met supplementation increases fat content in these conditions, but it could be due to its potential involvement in de novo fat synthesis or its ability to donate a methyl group for choline synthesis (National Research Council, 2001). There are still other studies in which the addition of RUP Lys and Met had no affect on milk fat yield or milk fat percentage (Donkin et al., 1989; Armentano et al., 1993; Bateman et al., 1999).

The effects of additional RUP Lys and Met on milk protein content are more consistent with several studies (Donkin et al., 1989; Armentano et al., 1997; Colin-Schoellen et al., 1995; Robinson et al., 1995; Piepenbrink et al., 1996; Xu et al., 1998) showing an increase in milk protein production and percentage when supplemental Lys and Met were fed. Xu et al. (1998) showed a 0.24 kg/d increase in protein yield from a high RUP Lys and Met diet over a negative control during the first eight weeks of the experiment. The increase in milk protein is economically important because true milk protein (mostly casein) is now factored in the price paid to dairy producers for their milk. Armentano et al. (1993) showed that feeding rumen protected Lys (16.6 g/d) and Met (5.6 g/d) would increase cheese yield by 300g/100kg of milk, which is a 3.5% increase in cheese yield.

Increasing milk fat, protein, and yield are not the only reasons for feeding M-RUP Lys and Met. The other reason is to increase N efficiency (i.e., conversion of N intake into milk N) by lactating cows to reduce N waste being excreted into the environment. However, it is possible for excessive M-RUP Lys and Met to be fed, leading to an inefficient use of N by the animal. Wright et al. (1998) found that urinary and fecal N excretion increased linearly with RUP supplementation. They found that 50% of N intake was converted to urinary N when cows were fed high RUP diets and that, as RUP increased, N utilization for milk N decreased. Wu and Satter (2000) agreed with Wright et al. (1998) that lower concentrations of RUP in the diet increased the efficiency with which the animal utilized N, where N efficiency is defined as the percentage of feed or absorbed N converted to milk N. However, high RUP levels will not always lead to decreased N efficiency. If the RUP supplies an EAA profile more in line with the animal requirements, then the N (protein) supplied by the RUP source can be used more efficiently.

Materials and Methods

Cows and Design

Forty multiparous and 22 primiparous Holsteins cows were used in a 2x2 factorial arrangement of treatments. Cows were randomly assigned to continuous 12-week treatments based on calving dates. Assignment to treatments was made 14 to 21 days postpartum. The week prior to assignment to treatment served as the covariate adjustment period during which measurements were taken for milk yield and composition, and body weight. Once half of the cows were assigned to treatments animals were assigned based on the average milk weights from the covariate period. This assured that the starting average milk weights across all four treatments were reasonably balanced. All cows were housed in tie stalls bedded twice daily with sawdust and had free access to water. Bovine somatotropin was administered beginning at 63 days postpartum for all cows.

Diets

Prior to freshening, all cows were fed a standard pre-fresh diet with the addition of a premix containing 5.5 g/d of rumen protected Met with soybean meal serving as the carrier. The post-fresh cows were fed twice daily ad libitum as a group until they were assigned to treatment. The post-fresh diet served as the covariate adjustment diet. It was balanced using the NRC (2001) ration balancing program for 1350 lbs. of body weight, 80.0 lbs./day of milk, 3.6% fat, and 2.9% milk true protein. The post-fresh diet consisted of 30% corn silage, 20% alfalfa hay, 4% whole cottonseed, 44.2% of a pelleted concentrate, and 0.8% of a premix containing supplemental metabolizable Met and Lys on a DM basis. The supplemental metabolizable Lys was from porcine blood meal and Smartamine M-L® (Aventis Animal Nutrition, Alpharetta, GA), which contains 40% Lys and 15% Met. The supplemental metabolizable Met was from Smartamine M-L® and Smartamine M® (Aventis Animal Nutrition, Alpharetta, GA), which contains 75% Met. According to the manufacturer both Smartamine® products are estimated to be 90% rumen undegradable. The post-fresh diet was designed to exceed metabolizable Lys and Met requirements to ensure that experimental treatment would not be initiated with animals coming out of severe amino acid deficiencies.

Treatment diets were arranged in a 2x2 factorial arrangement of 100% (**LoM-RUP**) or 110% (**HiM-RUP**) of the requirements stated by the NRC (2001), and (2) metabolizable Met and Lys supplementation at control levels (Lys at 6.15% of metabolizable protein (**MP**) and Met at 1.81% of MP) (**LoAA**): supplementation at 108% and 120% (**HiAA**) of control levels for metabolizable Lys and Met, respectively. Diets were balanced using the NRC (2001) ration balancing program for 1350 lbs. of body weight, 100 lbs./day of milk, 3.6% fat, and 3.9% milk true protein. Diets were fed twice daily at ad libitum intake. Daily feed intakes were recorded; feed offered was adjusted daily to allow for approximately 10% refusal. Dry matter of the diets was formulated to contain 30% corn silage, 15% alfalfa hay, 4% whole cottonseed, 49.2% of a pelleted concentrate, and 0.8% of a soybean meal premix containing the Met supplement or a placebo. The two unsupplemented diets were formulated to contain metabolizable Lys at 6.15% of MP, and metabolizable Met at 1.85% of MP. The two supplemented diets were balanced for Lys at 6.65%

Table 1. Calculated nutrient composition of diets.

	Treatments			
	LoM-RUP[1,3]		HiM-RUP[1,4]	
Nutrients[2]	LoAA[1,5]	HiAA[1,6]	LoAA	HiAA
NE_L (Mcal/kg)	1.61	1.60	1.62	1.62
CP (% of DM)	16.5	16.2	17.5	17.1
NDF (% of DM)	30.8	30.0	31.2	30.1
RDP (% of DM)	10.6	10.6	10.6	10.6
RUP (% of DM)	5.9	5.6	6.9	6.5
M-RUP (g/d)	1210	1210	1440	1440
Lys (% of MP)	6.17	6.68	6.15	6.65
Met (% of MP)	1.86	2.23	1.81	2.22
Lys:Met	3.32	3.00	3.40	3.00

[1] LoM-RUP = 100% of the M-RUP requirements stated by the NRC (2001).
HiM-RUP = 110% of M-RUP requirements stated by the NRC (2001).
LoAA = Lys and Met levels set at 6.15% and 1.81% of MP, respectively, and Lys:Met (3.3-3.4).
HiAA = Lys and Met levels set at 6.65% and 2.22% of MP, respectively, and Lys:Met (3.0).

[2] Calculated using computer model from NRC (2001).

[3] Low metabolizable-rumen undegradable protein

[4] High metabolizable-rumen undegradable protein

[5] Low Lys and Met concentrations and lower quality ratio

[6] High Lys and Met concentrations and higher quality ratio

Table 2. Ingredient and chemical composition of diets as a percentage of dry matter.

	Treatments			
	LoM-RUP[1,2]		HiM-RUP[1,3]	
Ingredients	LoAA[1,4]	HiAA[1,5]	LoAA	HiAA
	---% of total ration DM---			
Corn Silage	30	30	30	30
Alfalfa Hay	15	15	15	15
Ground corn	20	20	20	20
Barley	11.82	14.67	7.89	11.35
Soybean Meal, 44%	7.20	6.55	7.89	9.44
Soy Pass[6]	-	-	1.08	-
Corn distillers dried grains	2.23	-	4.64	-
Soybean hulls	4.00	4.00	4.00	4.00
Whole cottonseed	4.00	4.00	4.00	4.00
Porcine blood meal	-	1.46	0.54	2.20
Feather meal	1.50	-	0.85	-
Porcine meat and bone meal	0.50	-	0.97	-
Urea	0.12	0.31	-	0.04
Megalac[7]	0.81	0.81	0.81	0.81
Tallow	0.23	0.46	-	0.52
Smartamine M[8]	-	0.062	-	0.081
Vitamins and Minerals	4.63	4.86	4.05	4.72
Chemical composition				
DM, %	58.2	57.9	58.1	57.7
CP, %	16.0	15.9	16.4	16.5
NDF, %	31.5	31.1	32.3	31.5
ADF, %	20.6	20.4	20.8	20.8

[1] LoM-RUP = 100% of the M-RUP requirements stated by the National Research Council.
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LoAA = Lys and Met levels set at 6.15% and 1.81% of MP, respectively, and Lys:Met (3.3-3.4).
HiAA = Lys and Met levels set at 6.65% and 2.22% of MP, respectively, and Lys:Met (3.0).

[2] Low metabolizable-rumen undegradable protein

[3] High metabolizable-rumen undgradable protien

[4] Low Lys and Met concentrations and lower quality ratio

[5] High Lys and Met concentrations and higher quality ratio

[6] Rumen undegradable protein source

[7] Rumen protected fat source

[8] Rumen undegradable Met source containing 75% Met and 90% rumen undegradable

of MP, and Met at 2.25% of MP (Table 1). The supplemental metabolizable Lys was from porcine blood meal, whereas the supplemented metabolizable Met was from Smartamine M®. Rumen degradable protein, ether extract, and neutral detergent fiber (**NDF**) were kept the same across treatments at 10.6%, 4.6%, and 30% of DM, respectively. Diet composition is reported in Table 2.

Data Collection

Cows were milked twice daily at 0500h and 1800h. Milk production and composition data were collected starting with the covariate week and continued through the entire 12 weeks of the trial. Milk production was recorded for all milkings and was averaged weekly. Milk samples were taken weekly for four consecutive milkings and analyzed for fat, true protein, somatic cell count, lactose, and milk urea nitrogen (**MUN**) by Ohio DHI Inc., (Powell, OH).

Total mixed rations were sampled daily and composited weekly. Feed analyses for dry matter (**DM**), CP, NDF, and acid detergent fiber (**ADF**) were done on the weekly composites as well as weekly samples of the corn silage and alfalfa hay. Concentrates and whole cottonseed were sampled monthly and analyzed for DM, CP, NDF, and ADF by Dairy One Inc., (Ithaca, NY). Premixes were also sampled monthly and analyzed for Met inclusion rate and Met protection by Aventis Animal Nutrition, (Alpharetta, GA).

Body weights and body condition scores were determined weekly. Body condition scores were assessed on a five-point scale, with 1 being thin and 5 being very overconditioned animals (Wildmand et al., 1982). Body condition scores were determined independently by two individuals.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (2001) for a 2x2 factorial arrangement of treatments with covariate adjustments and repeated measures within cows. Residual errors were modeled using a first-order auto-regressive structure. The following model was used:

$Y_{ijklm} = \mu + R_i + A_j + RA_{ij} + P_k + RP_{ik} + AP_{jk} + RAP_{ijk} + B_k(X_{ijkl} - \bar{X}_k) + c_{ijkl} + W_m + RW_{im} +$
$AWjm + RAW_{ijm} + PW_{im} + RPW_{ikm} + APW_{jkm} + RAPW_{ijkm} + e_{ijkm}$

Where:

Y_{ijklm} is the dependent continuous variable

R_i is the fixed effect of the i^{th} M-RUP level, i = 1,2

A_j is the fixed effect of the j^{th} amino acid balance, j = 1,2

RA_{ij} is the fixed effect of i^{th} M-RUP level by j^{th} amino acid level interaction

P_k is the fixed effect of the k^{th} parity, k= 1,2

RP_{ik} is the fixed effect of i^{th} M-RUP level by k^{th} parity

AP_{jk} is the fixed effect of the j^{th} amino acid balance by k^{th} parity

RAP_{ijk} if the fixed effect of the i^{th} M-RUP level by j^{th} amino acid balance by k^{th} parity interaction

B_k is the k^{th} covariate regression coefficient

X_{ijkl} is the covariate measure of the l^{th} cow within the i^{th} M-RUP level, jth amino acid balance, and the k^{th} parity

$\bar{X}_k$ is the mean covariate measurement for the k^{th} parity

c_{ijkl} is the random effect of the l^{th} cow within the i^{th} M-RUP level, j^{th} amino acid balance, and the k^{th} parity

W_m is the fixed effect of the m^{th} week of experiment, m = 1,...,12

RW_{im} is the fixed effect of the i^{th} M-RUP level by m^{th} week interaction

AW_{jm} is the fixed effect of the j^{th} amino acid balance by m^{th} week interaction

RAW_{ijm} is the fixed effect of the i^{th} M-RUP level and j^{th} amino acid level by the m^{th} week of experiment interaction

PW_{km} is the fixed effect of the k^{th} parity by the m^{th} week of experiment interaction

RPW_{ikm} is the fixed effect of the i^{th} M-RUP level and k^{th} parity by the m^{th} week of experiment interaction

APW_{jkm} is the fixed effect of the j^{th} amino acid balance by k^{th} parity by the m^{th} week of experiment interaction

$RAPW_{ijkm}$ is the fixed effect of the i^{th} M-RUP level by j^{th} amino acid balance by k^{th} parity by the m^{th} week of experiment interaction

e_{ijklm} is the random residual error

Results and Discussion

Analyzed diet composition is reported in Table 2. Neutral detergent fiber and ADF concentrations remained relatively constant across treatments and throughout the experiment. The analyzed CP levels were lower than formulations due to slightly lower levels than expected in the corn silage and alfalfa hay. However, the CP levels were still high enough to meet estimated requirements. Dry matter intake (**DMI**) (Table 3) averaged 23.1 kg/d across diets. There was a significant effect of the interaction of M-RUP levels and parity on DMI. Multiparous cows had a greater DMI at higher M-RUP levels, whereas primiparous cows had a higher DMI at lower M-RUP levels ($P = 0.03$). Xu et al. (1998) and Robinson et al. (1995) observed an increase in DMI with increasing levels of RUP Lys and Met, but most studies agree with our findings that DMI is unaffected by levels of M-RUP or Lys and Met.

Milk yield was not significantly affected by M-RUP or Lys and Met levels (Table 3), but there was a trend ($P = 0.11$) towards an effect of the interaction of M-RUP with parity and week of experiment on milk yield. This trend shows that during some of the weeks in eary lactation multiparous animals responded to increased levels of M-RUP with increased milk yield while the primiparous cows showed no response. The higher than expected standard errors for milk yield, possibly due to outlier observations, may have prevented us from seeing greater significance. This is currently under investigation and statistical procedures for outlier detection in a mixed statistical model are being programmed. Numerically, cows on the HiM-RUP-HiAA diet produced more milk (1.5 kg/d), which agrees with the results from Robinson et al. (1995) in which Lys and Met fed at levels above the requirement

Table 3. Dry matter intake and milk production responses of cows fed different levels of M-RUP and Lys and Met supplementation.

	LoM-RUP[1,2]		HiM-RUP[1,3]			*P*-Values			
	LoAA[1,4]	HiAA[1,5]	LoAA	HiAA	SEM	M-RUP[6]	AA	Parity	M-RUP*Parity
DMI (kg/d)	**22.9**	**23.2**	**23.1**	**23.1**	**0.78**	**NS**	**NS**	**NS**	**0.033**
parity = 1	21.1	21.8	20.1	20.2	1.37				
parity = > 1	24.8	24.7	26.1	26.0	0.74				
Milk yield (kg/d)	**43.4**	**43.2**	**43.1**	**44.8**	**1.39**	**NS**	**NS**	**NS**	**NS**
parity = 1	39.8	40.3	41.1	40.9	2.44				
parity = > 1	47.0	46.0	46.0	48.8	1.30				
Fat production (kg/d)	**1.48**	**1.50**	**1.44**	**1.43**	**0.060**	**NS**	**NS**	**0.030**	**NS**
parity = 1	1.44	1.50	1.35	1.35	0.103				
parity = > 1	1.52	1.49	1.52	1.51	0.059				
Fat (%)	**3.34**	**3.39**	**3.21**	**3.07**	**0.106**	**0.047**	**NS**	**NS**	**NS**
parity = 1	3.43	3.59	3.17	3.05	0.167				
parity = > 1	3.26	3.19	3.26	3.09	0.129				
Protein production (kg/d)	**1.28**	**1.31**	**1.28**	**1.36**	**0.038**	**NS**	**0.078**	**0.077**	**NS**
parity = 1	1.19	1.19	1.16	1.23	0.066				
parity = > 1	1.38	1.43	1.40	1.50	0.036				
Protein (%)	**2.97**	**3.02**	**2.96**	**3.08**	**0.041**	**NS**	**0.049**	**NS**	**NS**
parity = 1	3.03	3.03	2.95	3.12	0.064				
parity = > 1	2.90	3.01	2.96	3.05	0.050				
Lactose production (kg/d)	**2.11**	**2.05**	**2.05**	**2.09**	**0.065**	**NS**	**NS**	**NS**	**NS**
parity = 1	1.94	1.92	1.90	1.90	0.111				
parity = > 1	2.28	2.19	2.20	2.28	0.066				
Lactose (%)	**4.90**	**4.78**	**4.81**	**4.73**	**0.024**	**0.006**	**<0.001**	**<0.001**	**NS**
parity = 1	4.95	4.85	4.84	4.77	0.038				
parity = > 1	4.86	4.72	4.77	4.70	0.029				

[1] LoM-RUP = 100% of the M-RUP requirements stated by the NRC (2001).
HiM-RUP = 110% of M-RUP requirements stated by the NRC (2001).
LoAA = Lys and Met levels set at 6.15% and 1.81% of MP, respectively, and Lys:Met (3.3-3.4).
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[6] Metabolizable-rumen undegradable protien

increased milk yield. Overall, milk yields were lower than the 42 to 48 kg/day that was expected based on similar research that was recently conducted at our facility. This could be due to lower quality corn silage than anticipated. The lower than expected milk yields could also have been due to substantial levels of mycotoxins (aflotoxin, 18.8 ppb; vomitoxin, 1.90 ppm) in the corn silage. Starting a month into this experiment, MTB-100 (Alltech, Nicholasville, KY) was added at a rate of 4.0 lbs/ton of pelleted concentrate to each of the four diets. The overall health of the animals seemed to improve, but the additive does not claim 100% efficiency at binding mycotoxins. Thus, residual effects of these toxins were most likely present.

There was a significant effect of higher Lys and Met levels (HiAA) on true protein production ($P < 0.10$) and milk true protein percentage ($P < 0.10$). This confirms our hypothesis that providing higher levels of metabolizable Lys and Met and in a better ratio would lead to higher protein concentration. This also agrees with the results of numerous studies that have shown that increasing metabolizable Lys and Met increases milk true protein production. However, we hypothesized that adding additional Lys and Met to a diet already high in M-RUP would not increase protein concentration to the same extent as when a diet contains a more moderate level of M-RUP. Numerically, our observations are in line with this hypothesis, but statistically significance cannot be claimed ($P < 0.10$).

Milk fat percentage was significantly higher for the LoM-RUP diets. Higher levels of Lys and Met did not have a significant effect on milk fat production or concentration. Other studies showed either no effect (Armentano et al. 1993) or increased milk fat content from higher levels of Met in diets (Overton et al., 1996; Robinson et al., 1998).

The percent lactose was increased by the LoM-RUP-LoAA diet over the other three. Lactose is one of primary osmotic regulator in the mammary gland. Thus, it is uncommon to see a significant effect of dietary treatments on lactose concentration of milk. This effect on lactose concentration, like the effect on fat, may be due to differences in milk production for animals on the LoM-RUP diets.

Estimated N efficiencies are shown in Table 4. These N efficiencies were calculated using the models developed by Kauffman and St-Pierre (2001). Lower levels of M-RUP ($P = 0.003$) and lower amino acid levels ($P = 0.02$) significantly reduced urinary N excretion. Overall, the cows on the HiM-RUP-HiAA diets had higher N intakes and excreted 25 g/d more urinary N than the cows on the LoM-RUP-HiAA diet. However, cows were numerically more efficient at converting intake N to milk N at higher amino acid levels with Lys to Met ratios closer to the NRC (2001) recommendation. Parity had a significant impact on N intake, N digested, milk N/N intake (gross N efficiency), and N excreted/milk N (environmental efficiency). Primiparous cows had higher N intakes, less N digested and higher fecal N excretions on the LoM-RUP diets. Multiparous cows on the LoM-RUP diets had lower N intakes, lower N digested, and lower N excretion in feces and urine than multiparous cows on the HiM-RUP diets. Overall, multiparous cows were more efficient at converting intake N to milk N and excreted less N per unit of milk N produced than primiparous cows. Multiparous cows in general use feed more efficiently for milk production over primiparous animals because they do not have to

Table 4. Nitrogen efficiencies of cows fed two different levels of M-RUP and supplementation of Met and Lys.

	LoM-RUP[1,2]		HiM-RUP[1,3]			*P*-Values					
	LoAA[1,4]	HiAA[1,5]	LoAA	HiAA	SEM	M-RUP[6]	AA	Parity	Parity*M-RUP	Parity*AA	Parity*M-RUP*AA
N intake (g/d)	**584**	**591**	**602**	**608**	**15.7**	**NS**	**NS**	**<.001**	**0.032**	**NS**	**NS**
parity = 1	516	526	498	507	24.7						
parity > 1	653	656	706	709	19.3						
N digested (g/d)	**368**	**373**	**376**	**394**	**5.6**	**0.018**	**0.057**	**<.001**	**NS**	**NS**	**NS**
parity = 1	322	329	331	338	8.7						
parity > 1	415	416	421	451	7.0						
Urinary N (g/d)	**168**	**175**	**179**	**193**	**4.1**	**0.003**	**0.024**	**NS**	**NS**	**NS**	**NS**
parity = 1	153	168	170	181	6.4						
parity > 1	184	181	188	205	4.4						
Milk N production (g/d)	**201**	**206**	**201**	**214**	**5.9**	**NS**	**0.078**	**0.077**	**NS**	**NS**	**NS**
parity = 1	187	187	183	192	10.3						
parity > 1	216	224	219	235	2.7						
Gross N efficiency (%)[7]	**34.2**	**34.4**	**33.0**	**34.7**	**0.75**	**NS**	**NS**	**0.003**	**NS**	**NS**	**NS**
parity = 1	33.1	31.9	32.6	33.8	1.19						
parity > 1	35.4	36.9	33.4	35.6	0.93						
Environmental efficiency[8]	**2.00**	**2.00**	**2.10**	**1.90**	**0.058**	**NS**	**NS**	**0.003**	**0.075**	**0.055**	**NS**
parity = 1	2.00	2.20	2.10	2.00	0.092						
parity > 1	1.90	1.80	2.10	1.90	0.072						
MUN (mg/d)	**10.5**	**10.6**	**11.7**	**12.4**	**0.20**	**<0.001**	**0.042**	**NS**	**NS**	**NS**	**0.025**
parity = 1	10.3	11.0	12.1	12.5	0.31						
parity > 1	10.7	10.2	11.4	12.4	0.24						

[1] LoM-RUP = 100% of the M-RUP requirements stated by the National Research Council.
HiM-RUP = 110% of M-RUP requirements stated by the National Research Council.
LoAA = Lys and Met levels set at 6.15% and 1.81% of MP, respectively, and Lys:Met (3.3-3.4).
HiAA = Lys and Met levels set at 6.65% and 2.22% of MP, respectively, and Lys:Met (3.0).
[2] Low metabolizable-rumen undegradable protein
[3] High metabolizable-rumen undegradable protein
[4] Low Lys and Met concentrations and lower quality ratio
[5] High Lys and Met concentrations and higher quality ratio
[6] Metabolizable-rumen undegradable protein
[7] kg milk N/g N intake * 100
[8] kg N excreted/kg N in milk

divert nutrients from milk production to growth. Based on previous studies (Wright et al., 1998;Wu and Satter, 2000), we expected that the animals on the LoM-RUP-HiAA diet would have been the most efficient because the diet contained sufficient metabolizable protein to meet the calculated requirement but had additional metabolizable Lys and Met in the proper ratio to enhance milk protein secretion. The significantly lower MUN concentrations for the LoM-RUP diets do indicate that the N was used more efficiently than in the HiM-RUP diets.

Dietary treatments had a significant interaction effect with parity on body weight and body condition score. Primiparous cows on the LoM-RUP-LoAA diet showed a significantly greater rate of increase in body condition score during the first twelve weeks of the experiment ($y = 0.03x + 3.2$, $r^2 = 0.81$, $P < 0.001$). Multiparous cow were unaffected by treatments. There was also a significant effect of M-RUP levels on body weight. Animals on the LoM-RUP diets averaged 633 kg while animals on the HiM-RUP diets averaged 618 kg. Again, this may indicate that the LoM-RUP diets led to slightly lower milk production, especially in primiparous cows, allowing more energy to go into growth and fat deposition. However, without doing more analyses to determine energy balance it is hard to know exactly what is causing this effect.

Conclusions

Supplemental metabolizable Lys and Met provided in the proper profile significantly increased milk true protein production, daily amounts of N digested, and urinary N excretion. Decreasing the M-RUP levels in the diet decreased urinary N excretion and increased milk fat content. M-RUP levels had no effect on milk yield or milk components except for milk fat percentage, which indicates that the M-RUP levels recommended by the NRC (2001) may not be predicting M-RUP requirements accurately. However, supplementation with metabolizable Lys and Met led to a significant increase in protein concentration in the diet, which supports the NRC (2001) recommendations. This study supports many of the findings of previous research, but there is still a lot of variability of the effects of M-RUP and metabolizable Lys and Met levels on N efficiency and milk production parameters. More research is needed in this area to determine more precisely optimum M-RUP, Lys, and Met levels needed to maximize milk yield and component production without causing detrimental effects on the environment.

Definition of Terms

Body condition score- A measurement of the subcutaneous body fat in the cow.

Degradation- The breaking down of material in the rumen by the rumen microbes.

Essential amino acids- Amino acids which can not be made by the body or can not be made in sufficient quantities to meet the animals needs.

Freshening- Calving

Metabolizable protein- Protein that is able to be absorbed by the intestine.

Multiparous- Cows that are in their second or higher lactation.

Mycotoxins- Toxins produced by fungi that can grow in forage sources.

Primiparous- Cows that are in their first lactation.

Neutral detergent fiber- Laboratory analysis that measure the hemicellulose, cellulose, and lignin content of feed.

Rumen undegradable protein- Protein that can bypass the rumen relatively unchanged.

Literature Cited

1. Armentano, L. E., S. J. Bertics, and G. A. Ducharme. 1997. Response of lactating cows to methionine or methionine plus lysine added to high protein diets based on alfalfa and heated soybeans. J. Dairy Sci. 80:1194-1199.

2. Armentano, L. E., S. M. Swain, and G. A. Ducharme. 1993. Lactation response to ruminally protected methionine and lysine at two amounts of ruminally available nitrogen. J. Dairy Sci. 76:2963-2969.

3. Bach, A., G. B. Huntington, S. Calsamiglia, and M. D. Stern. 2000. Nitrogen metabolism of early lactation cows fed diets with two different levels of protein and different amino acid profiles. J. Dairy Sci. 83:2585-2595.

4. Bateman, H. G., J. N. Spain, M. S. Kerley, R. L. Belyea, and R. T. Marshall. 1999. Evaluation of ruminally protected methionine and lysine or blood meal and fish meal as protein sources for lactating Holsteins. J. Dairy. Sci. 82: 2115-2120.

5. Colin-Schoellen, O. F. Laurent, B. Vignon, J. C. Robert, and B. Sloan. 1995. Interaction of ruminally protected methionine and lysine with protein source or energy level in the diets of cows. J. Dairy Sci. 78:2807-2818.

6. Cunningham, K. D., M. J. Cecava, T. R. Johnson, and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79:620-630.

7. Donkin, S. S., G. A. Varga, T. F. Sweeney, and L. D. Muller. 1989. Rumen-protected methionine and lysine: effects on animal performance, milk protein yield, and physiological measures. J. Dairy Sci. 72:1484-1491.

8. Kauffman, A. J. and N. R. St-Pierre. 2001. The relationship of milk urea nitrogen excretion in Holstein and Jersey cows. J. Dairy Sci. 84:(accepted).

9. Meisinger, J. J., and W. E. Jokela. 2000. Ammonia losses from manure. Page 109-115. Cornell Nutr. Conf., Ithaca, NY.

10. National Research Council. 2001. Nutritional requirements of dairy cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

11. Nichols, J. R., D. J. Schingoethe, H. A. Maiga, M. J. Brouk, and M. S. Piepenbrink. 1998. Evaluation of corn distillers grains and ruinally protected lysine and methionine for lactating dairy cows. J. Dairy Sci. 81:482-491

12. Overton, T. R., D. W. LaCount, T. M. Cicela, and J. H. Clark. 1996. Evaluation of Ruminally Protected Methionine Product for Lactating Dairy Cows. J. Dairy Sci. 79:631-638.

13. Piepenbrink, M. S., T. R. Overton, and J. H. Clark. 1996. Response of cows fed a low crude protein diet to ruminally protected methionine and lysine. J. Dairy Sci. 79:1638-1646.

14. Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acid in growing cattle. J. Anim. Sci. 46:740-745.

15. Robinson, P. H., W. Chalupa, C. J. Sniffen, W. E. Julien, H. Sato, K. Watanabe, T. Fujieda, and H. Suzuki. 1998. Ruminally Protected Lysine or Lysine and Methionine for lactating Dairy Cows Fed a Ration Designed to Meet Requirements for Microbial and Postruminal Protein. J. Dairy Sci. 81:1364-1373.

16. Robinson, P. H., A. H. Fredeen, W. E. Julien, H. Sato, T. Fujieda, and H. Suzuki. 1995. Ruminally protected lysine and methionine for lactating dairy cows fed a diet designed to meet requirements for microbial and postruminal protein. J. Dairy Sci. 78:582-594.

17. SAS/STAT User's Guide, Version 8.1 Edition. 2001. SAS Inst., Inc., Cary, NC.

18. Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M. M. A. Mesbah. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. sequence of lysine and methionine limitation. J. Dairy Sci. 75:3486-3502.

19. St-Pierre, N. R. and C. S. Thrain. 1999. Animal grouping strategies, sources of variation, and economic factors affecting nutrient balance on dairy farms. J. Dairy Sci. 82(Suppl. 2) 72-83.

20. Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

21. Wright, T. C., S. Moscardini, P. H. Luimes, P. Susmel, and B. W. McBride. 1998. Effects of Rumen-Undegradable Protein and Feed Intake on Nitrogen Balance and Milk Protein Production in Dairy Cows. J. Dairy Sci. 81:784-793.

22. Wu, Z., R. J. Fisher, C. E. Polan, and C. G. Schwab. 1997. Lactational performance of cows fed low or high ruminally undegradable protein prepartum and supplemental methionine and lysine postpartum. J. Dairy Sci. 80:722-729.

23. Wu, Z., and L. D. Satter. 2000. Milk Production During the Complete Lactation of Dairy Cows Fed Diets Containing Different Amounts of Protein. J. Dairy Sci. 83:1042-1051.

24. Xu, S., J. H. Harrison, W. Chalupa, C. Sniffen, W. Julien, H. Sato, T. Fujieda, K. Watanabe, T. Ueda, and H. Suzuki. 1998. The Effect of Ruminal Bypass Lysine and Methionine on Milk Yield and Composition of Lactating Cows. J. Dairy Sci. 81:1062-1077.